Cardioprotection afforded by chronic exercise is mediated by the sarcolemmal, and not the mitochondrial, isoform of the K_{ATP} channel in the rat

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This study was conducted to examine the role of myocardial ATP-sensitive potassium (K_{ATP}) channels in exercise-induced protection from ischaemia–reperfusion (I–R) injury. Female rats were either sedentary (Sed) or exercised for 12 weeks (Tr). Hearts were excised and underwent a 1–2 h regional I–R protocol. Prior to ischaemia, hearts were subjected to pharmacological blockade of the sarcolemmal K_{ATP} channel with HMR 1098 (SedHMR and TrHMR), mitochondrial blockade with 5-hydroxydecanoic acid (5HD; Sed5HD and Tr5HD), or perfused with buffer containing no drug (Sed and Tr). Infarct size was significantly smaller in hearts from Tr animals (35.4 ± 2.3 *versus* 44.7 ± 3.0% of the zone at risk for Tr and Sed, respectively). Mitochondrial K_{ATP} blockade did not abolish the training-induced infarct size reduction (30.0 ± 3.4 *versus* 38.0 ± 2.6 in Tr5HD and Sed5HD, respectively); however, sarcolemmal K_{ATP} blockade completely eradicated the training-induced cardioprotection. Infarct size was 71.2 ± 3.3 and 64.0 ± 2.4% of the zone at risk for TrHMR and Sed HMR. The role of sarcolemmal K_{ATP} channels in Tr-induced protection was also supported by significant increases in both subunits of the sarcolemmal K_{ATP} channel following training. LV developed pressure was better preserved in hearts from Tr animals, and was not influenced by addition of HMR 1098. 5HD decreased pressure development regardless of training status, from 15 min of ischaemia through the duration of the protocol. This mechanical dysfunction was likely to be due to a 5HD-induced increase in myocardial Ca^{2+} content following I–R. The major findings of the present study are: (1) unlike all other known forms of delayed cardioprotection, infarct sparing following chronic exercise was not abolished by 5HD; (2) pharmacological blockade of the sarcolemmal K_{ATP} channel nullified the cardioprotective benefits of exercise training; and (3) increased expression of sarcolemmal K_{ATP} channels was observed following chronic training.

(Received 1 August 2005; accepted after revision 10 October 2005; first published online 13 October 2005)
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The phenomenon of myocardial preconditioning has been extensively studied since its discovery by Murry and colleagues (Murry *et al.* 1986). Cardiac preconditioning (PC) can be triggered by a number of different stimuli and has been shown to reduce infarct size and defend against myocardial stunning. Protection afforded by PC appears to be biphasic in nature, with an initial window of protection (referred to as 'classic PC') lasting 1–2 h after the PC stimulus, and a second window of protection (referred to as 'late' or 'delayed PC'), which occurs 24–72 h following the PC stimulus (see Yellon & Downey, 2003 for review). While many factors appear to be potent in reducing myocardial infarct size when administered acutely, repetitive administration of the preconditioning stimuli such as ischaemic PC (Cohen *et al.* 1994; Tsuchida *et al.* 1994) or adenosine receptor agonist (Tsuchida *et al.* 1994) results in a loss of efficacy, indicative of a tachyphylactic response. Therefore, the clinical applicability of these stimuli as infarct-sparing strategies continues to be in question (Yellon & Downey, 2003).

A single bout of exercise training has been shown to protect the myocardium against ischaemia–reperfusion (I–R) damage in a biphasic manner (Yamashita *et al.* 1999) consistent in amplitude and temporal onset as

 DOI: 10.1113/jphysiol.2005.095729

that afforded by other PC stimuli (Bolli, 2000; Yellon & Downey, 2003). Subsequent to the findings of Yamashita *et al.* (1999), our laboratory and others have demonstrated that the delayed phase of PC following exercise can be sustained after days (Yamashita *et al.* 2001; Hamilton *et al.* 2003; Brown *et al.* 2005) or months (Brown *et al.* 2003) of training. Other than chronic moderate ethanol consumption (Kehl *et al.* 2003), exercise training is the only stimulus demonstrated to sustain myocardial protection against infarction over such an extended period of time. This finding continues to be extremely relevant from a clinical standpoint, as myocardial infarction affects over 1 million Americans annually (National Health and Nutrition Examination Survey III, 2000), and size of infarction correlates inversely with chance of both short-term (Miller *et al.* 1995) and long-term survival (Herlitz *et al.* 1988).

Our previous work indicated that 20 weeks of exercise training significantly reduced myocardial infarct size after *ex vivo* I–R 24–36 h after the last bout of exercise (Brown *et al.* 2003). We postulated that improved vascular reactivity and increased manganese superoxide dismutase protein expression were responsible for the infarct sparing that was observed following exercise, although subsequent experiments have demonstrated that protection from infarction can be accomplished in the absence of improvements in these variables following short-term exercise (Brown *et al.* 2005). While the tissue-salvaging effect of exercise seems evident, the terminal mechanism(s) evoking this protective phenotype remain(s) to be elucidated.

The role of cardiac ATP-sensitive potassium (K_{ATP}) channels in preconditioning has received much attention over the last 10 years, with investigations exploring the protective role of both the sarcolemmal ($sarcK_{ATP}$) and the mitochondrial ($mitoK_{ATP}$) isoforms of this channel (for review see O'Rourke, 2000; Baxter & Ferdinandy, 2001; Yellon & Downey, 2003; O'Rourke, 2004). Several investigations have implicated a distal role for the K_{ATP} channel in delayed protection afforded by a variety of stimuli (Bolli, 2000); however, only one study has examined the role of the K_{ATP} channels in exercise-induced protection from infarction. Data from our laboratory indicated that short-term exercise led to reduced infarct size following I–R, with the delayed protection correlating closely with increased expression of the $sarcK_{ATP}$ channel in both males and females (Brown *et al.* 2005). Although we observed a close relationship between $sarcK_{ATP}$ protein expression and protection from I–R injury, pharmacological blockade of this channel population to confirm the postulated channel-mediated cardioprotection was not performed in our previous work (Brown *et al.* 2005). In the present study, we hypothesized that the increased $sarcK_{ATP}$ channel expression would be sustained following months of training, and that pharmacological blockade of this channel population would abolish the protection from infarction afforded by training.

There is also evidence from the literature that delayed PC induced by a variety of stimuli is comprehensively abolished by 5-hydroxydecanoic acid (5HD), a putative mitochondrial ATP-sensitive K^+ ($mitoK_{ATP}$) channel antagonist, administered minutes before index ischaemia (Pell *et al.* 1997; Baxter & Yellon, 1999; Bernardo *et al.* 1999; Fryer *et al.* 1999; Ockaili *et al.* 2001, 2002). These experiments provide compelling evidence for a role of the $mitoK_{ATP}$ channel as a distal mediator of protection from infarction. As such, our interest in exercise-induced PC led us to hypothesize that $mitoK_{ATP}$ channels may also be integrally involved in delayed protection following exercise training, and that administration of 5HD would abrogate training-induced reductions in myocardial infarct size following I–R. Therefore, the present study sought to determine if blockade of either the sarcolemmal and/or mitochondrial isoforms of the K_{ATP} channel abolished the protection afforded by chronic exercise training.

Methods

Animal model

Adult female Sprague–Dawley rats (Harlan; $n = 103$ total animals) were used in the study. Experiments were conducted with prior approval from the Institutional Animal Care and Use Committee at the University of Colorado at Boulder, and in accordance with guidelines established by the American Physiological Society.

I–R protocol

Animals were trained for $\geq$12 weeks, as previously described (Brown *et al.* 2003). At the time of death, animals were anaesthetized with sodium pentobarbital (35 mg kg^{-1}; I.P. injection) and hearts were excised and very rapidly hung by the aorta to a cannula of a modified Langendorff apparatus. Both left and right adrenal glands were removed and weighed, and plantaris muscle was dissected for analysis of citrate synthase activity (Brown *et al.* 2003). Retrograde perfusion was initiated using an established buffer (Brown *et al.* 2003, 2005), and a 3-F pressure-transducing catheter (Millar) was simultaneously inserted into the left ventricle (LV) via the aortic valve for the collection of left-ventricular developed pressure (LVDP) waveforms as previously described (Brown *et al.* 2003, 2005). All chemicals were obtained from Sigma-Aldrich unless otherwise noted. After a 10 min equilibration period, baseline measurements of LVDP were obtained. Following the baseline measurements, hearts from trained and sedentary animals were divided further into a total of six groups (animal numbers represent

numbers used in data analysis): hearts to be perfused for the duration of the protocol with control buffer containing no K_{ATP} channel blocker (Sed and Tr; $n = 19$ Sed and 15 Tr), hearts to be perfused for the duration of the protocol with sarcolemmal K_{ATP} blocker (30 μM HMR 1098; a gift from Dr Heinz Gögelein, Aventis Pharma, Deutschland) in the perfusate (SedHMR and TrHMR; $n = 9$ each), and hearts perfused with mitochondrial K_{ATP} blocker (100 μM 5HD) for the duration of the protocol (Sed5HD and Tr5HD; $n = 14$ Sed5HD 13 Tr5HD). The concentrations of HMR 1098 and 5HD were previously used in both Langendorff-perfused rat heart (Chen *et al.* 2003; D'Souza *et al.* 2003; Kristiansen *et al.* 2005) and single-cell experiments (Light *et al.* 2001), and this concentration of 5HD was effective in abolishing the infarct-sparing effects of classic ischaemic preconditioning. A separate group of hearts underwent three bouts of 5 min alternating regional I–R before index ischaemia, and the reduction in infarct size following ischaemic preconditioning ($5.4 \pm 2.6\%$ of the zone at risk) was abolished when hearts received 5HD during the ischaemic preconditioning (IPC) stimulus ($22.6 \pm 2.7\%$ of the zone at risk; $P < 0.05$). LVDP measurements were taken after five additional minutes of exposure to K_{ATP} channel blockers (or control buffer) before regional ischaemia was initiated. The I–R protocol, infarct size assessment, and haemodynamic measurement time points were identical to methods previously described by our laboratory (Brown *et al.* 2003, 2005).

Non-ischaemic time controls

We subjected a separate group of hearts to the 3 h perfusion protocol in the absence of ischaemia to determine the effect of the crystalloid perfusate and pharmacological agents on haemodynamic parameters and infarct size.

Western blotting

A separate group of Tr and Sed animals ($n = 9$ Tr and 9 Sed) were anaesthetized and hearts were excised. LV free wall was isolated and rinsed in saline (4°C), and homogenized and probed for $K_{ir}6.2$, SUR2a, Akt, p-Akt, glycogen synthase kinase (GSK)-3β, and p-GSK-3β as previously described (Brown *et al.* 2003, 2005; and Watson *et al.* manuscript currently in review).

Intracellular Ca^{2+} content

A separate group of hearts from Tr and Sed animals ($n = 36$ total) were exposed to a truncated I–R protocol (30 min each) in the presence or absence of the experimental drugs, and subsequently analysed for intracellular Ca^{2+} content as previously described (Alto & Dhalla, 1979). This abbreviated I–R protocol did not elicit measurable infarct sizes in experimental hearts (data not shown).

Exclusion criteria

Data were omitted from analysis if one of the following criteria were met: unclear resolution of heart slice images precluded analysis of infarction ($n = 1$); coronary flow did not decrease at the onset of ischaemia or increase at the onset of reperfusion (indicative of inefficient suture placement; $n = 14$); or hearts did not complete the I–R protocol due to excessive fibrillation or technical difficulty ($n = 8$). One animal died of natural causes during the course of the study.

Statistical analyses

Data are presented as means ± standard error. All statistical analyses were performed using SPSS Software, and α-level was predetermined to be $P < 0.05$. *A priori* confirmatory comparisons of infarct size and LVDP between Sed and Tr groups were performed with a two-tailed Student's *t* test. All other infarct size comparisons were performed with a 2×3 ANOVA (training group × drug). The LV, body, spleen and adrenal gland weights, and citrate synthase activity data, were pooled according to training status and analysed with a two-tailed Student's *t* test (all Tr *versus* all Sed). A repeated-measures ANOVA was employed for analysis of LVDP during ischaemia and again following the onset of reperfusion. Main effects of drug were analysed at each time point using a one-way ANOVA with a Bonferroni correction for *post hoc* comparisons.

Results

Morphology

Morphological data are presented in Table 1. Rats in the Tr group had significantly greater body weights, LV weights, and plantaris muscle citrate synthase activity than Sed animals ($P < 0.05$). A resting bradycardia was observed following chronic training, with Tr animals having a lower resting heart rate than Sed prior to the onset of ischaemia. There were no differences in adrenal weights between the groups, and there was a subtle increase in spleen weight with training, indicating that the exercise protocol did not elicit classic markers of a systemic stress response (Selye, 1998; Moraska *et al.* 2000).

Infarct size

Infarct sizes and representative images are presented in Fig. 1. The mean zone at risk (ZAR) for each experimental

Table 1. Morphological characteristics for rats used in the study

	Sed	Tr	*P* value
Body (g)	258 ± 3	273 ± 3*	0.001
Left ventricle (g)	0.620 ± 0.010	0.709 ± 0.012*	<0.00001
Baseline HR (beats min^{-1})	246 ± 6	221 ± 11*	0.04
Left adrenal (g cm^{-1} ($\times 10^4$))	8.3 ± 0.2	8.6 ± 0.2	NS
Right adrenal (g cm^{-1} ($\times 10^4$))	7.9 ± 0.2	8.3 ± 0.2	NS
Spleen (g cm^{-1} ($\times 10^3$))	16.2 ± 0.4	17.3 ± 0.4*	0.04
Citrate synthase (μmol g^{-1} min^{-1})	7.3 ± 0.5	10.1 ± 0.9*	0.01

group ranged from 42 to 46% of the LV, with no between-group differences present ($P =$ NS). Chronic exercise training led to a significant reduction in infarct size, with the Tr and Sed animals having infarct sizes of 35.4 ± 2.3 and 44.7 ± 3.0, respectively ($P < 0.01$, Sed *versus* Tr). Exercise-induced reductions in infarct size persisted in the presence of 5HD, with Tr5HD and Sed5HD groups having infarct sizes of 30.0 ± 3.4 and 38.0 ± 2.6, respectively ($P < 0.05$). Importantly, the addition of HMR 1098 abolished the infarct size difference between Tr and Sed, with the TrHMR hearts showing a trend for increased infarct size *versus* SedHMR ($P = 0.088$). The presence of HMR 1098 led to significant increases in infarct size in both the TrHMR (71.2 ± 3.3%) and SedHMR (64.0 ± 2.4%) groups when compared with respective controls ($P < 0.001$), with the magnitude of the increase from control being much greater in the TrHMR when compared to SedHMR (100% increase in infarct size

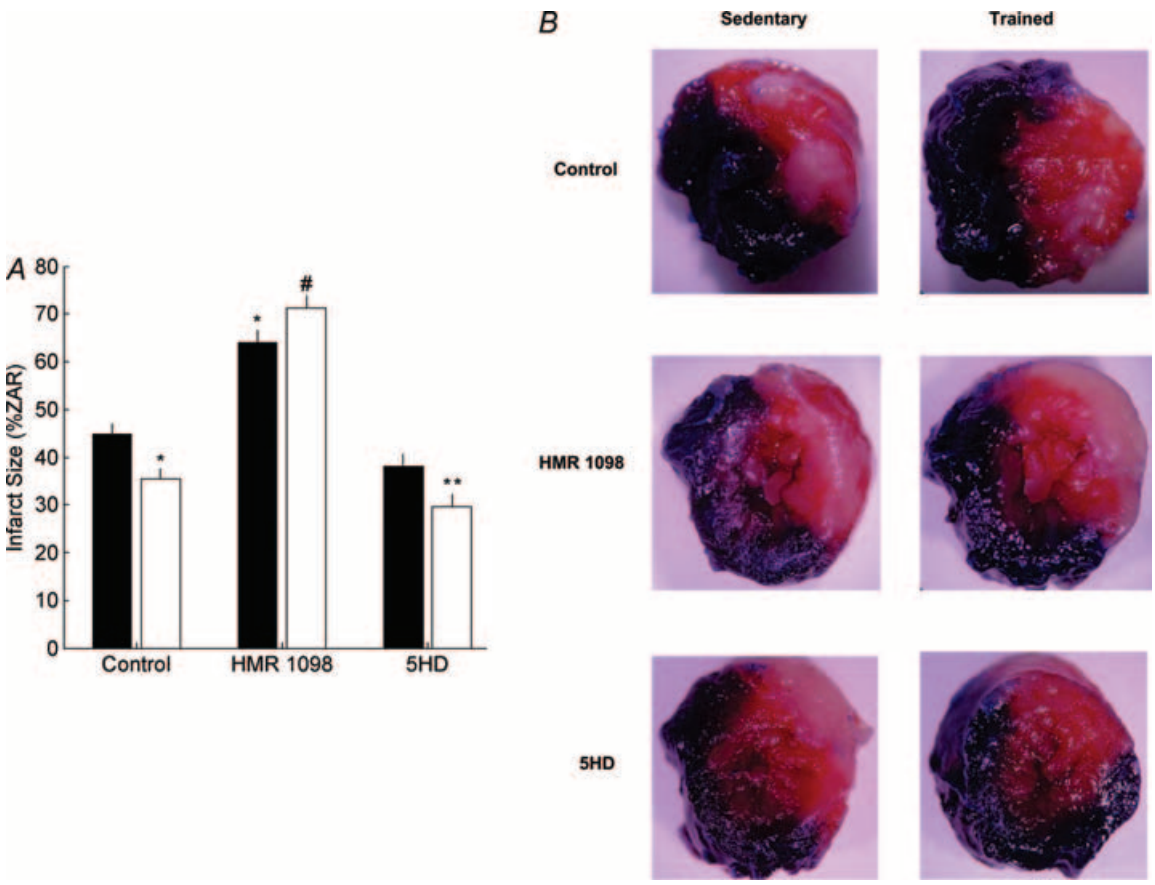

Figure 1. Myocardial Infarct Size
A, infarct size (expressed as a function of the zone at risk, ZAR) for experimental groups in the study. Filled bars represent hearts from sedentary animals, and open bars represent hearts from trained animals. Data are separated according to pharmacological agent perfused in the buffer throughout the protocol, with control buffer having no channel blocker, mitochondrial K_{ATP} channel blockade with 5-hydroxydecanoic acid (5HD), or sarcolemmal-specific K_{ATP} channel blocker HMR 1098. $^*P < 0.05$ *versus* sedentary control, $^{**}P < 0.05$ *versus* sedentary 5HD, #$P < 0.05$ *versus* trained control. *B*, representative images of left ventricular slices from the experimental groups in the study.

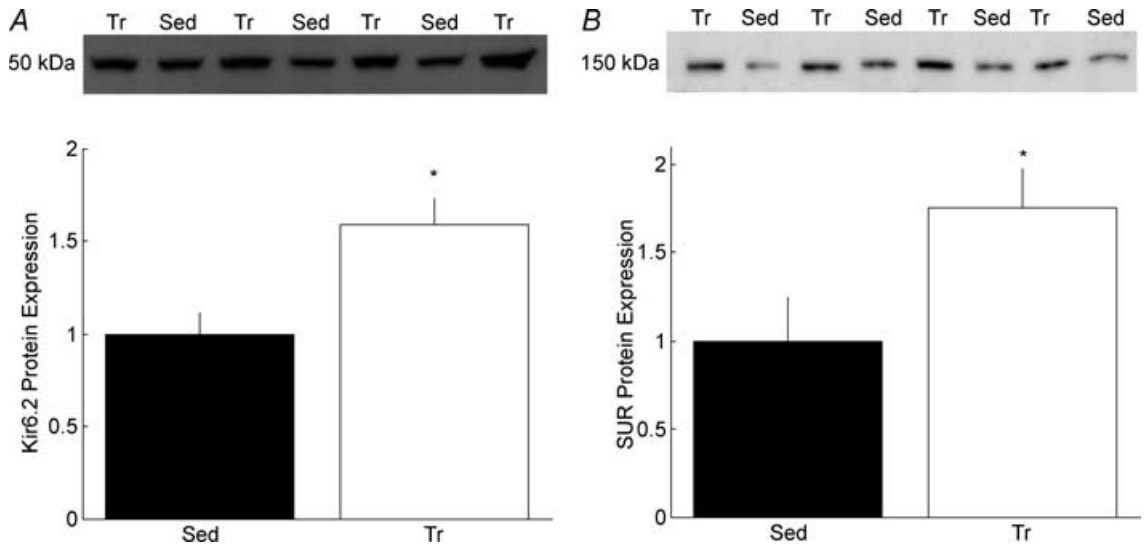

Figure 2. Myocardial ATP-sensitive K^+ (K_{ATP}) channel subunit expression for trained (Tr) and sedentary (Sed) animals
A, representative bands and quantification for the pore-forming subunit of the K_{ATP} channel, $K_{ir}6.2$. *B*, representative bands and quantification for the sulphonylurea receptor (SUR), the regulatory subunit of the K_{ATP} channel. * $P < 0.05$ *versus* Sed.

in Tr *versus* 43% increase in Sed). Administration of either drug alone did not induce significant infarction, with non-ischaemic time controls having infarct sizes of 2.5 ± 0.6, 2.4 ± 0.4 and 3.7 ± 1.5% of the ZAR for the control, 5HD and HMR groups, respectively. There were no differences in infarct size between the non-ischaemic time-control groups.

K_{ATP} channel expression

Protein expression of K_{ATP} channel subunits is presented in Fig. 2. Expression of the pore-forming subunit $K_{ir}6.2$ increased by 58% in the Tr groups ($P < 0.05$; Fig. 2*A*). Protein content of the sulphonylurea receptor (SUR) subunit was increased by 75% in hearts of animals that were trained ($P < 0.05$; Fig. 2*B*).

LV mechanical performance

LVDP data are presented in Fig. 3. Hearts from Tr animals maintained LVDP better than Sed animals from 30 min into ischaemia through the first 5 min of reperfusion (Fig. 3). A significant main effect of drug was observed both during ischaemia and through the course of

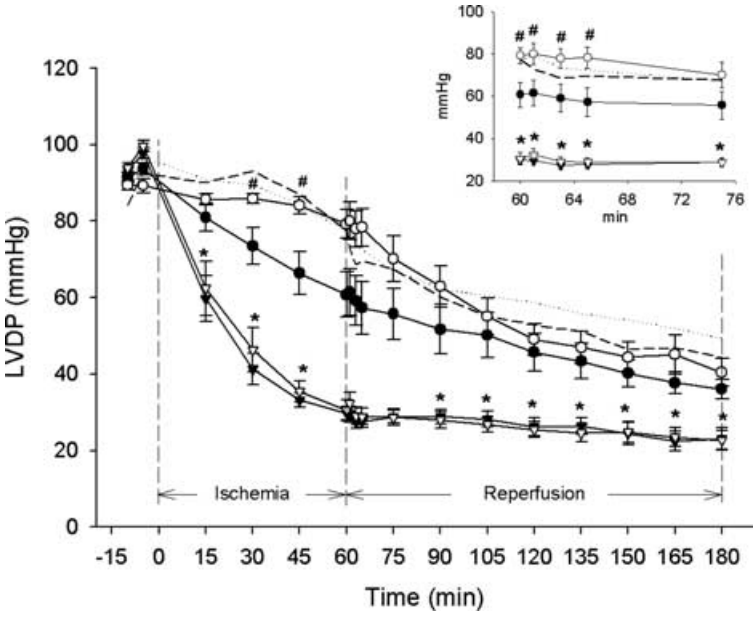

Figure 3. Left ventricular developed pressure (LVDP) for Langendorff-perfused hearts
●, sedentary control hearts (no channel blocker in the buffer); ○, trained control hearts (no channel blocker in the buffer); ▼ and ▽, sedentary and trained hearts, respectively, exposed to buffer with 5HD, a mitochondrial K_{ATP} channel blocker. #$P < 0.05$ *versus* sedentary control hearts, *$P < 0.05$ *versus* all other hearts exposed to either no drug or HMR 1098, a selective sarcolemmal K_{ATP} channel antagonist. Since there were no differences in developed pressure between control hearts and those that received HMR 1098, LVDP for hearts exposed to HMR 1098 are presented as lines for sedentary (dashed line) and trained (dotted line) animals in the interest of presentational clarity. Inset, LVDP for the first 15 min of reperfusion on an expanded time scale.

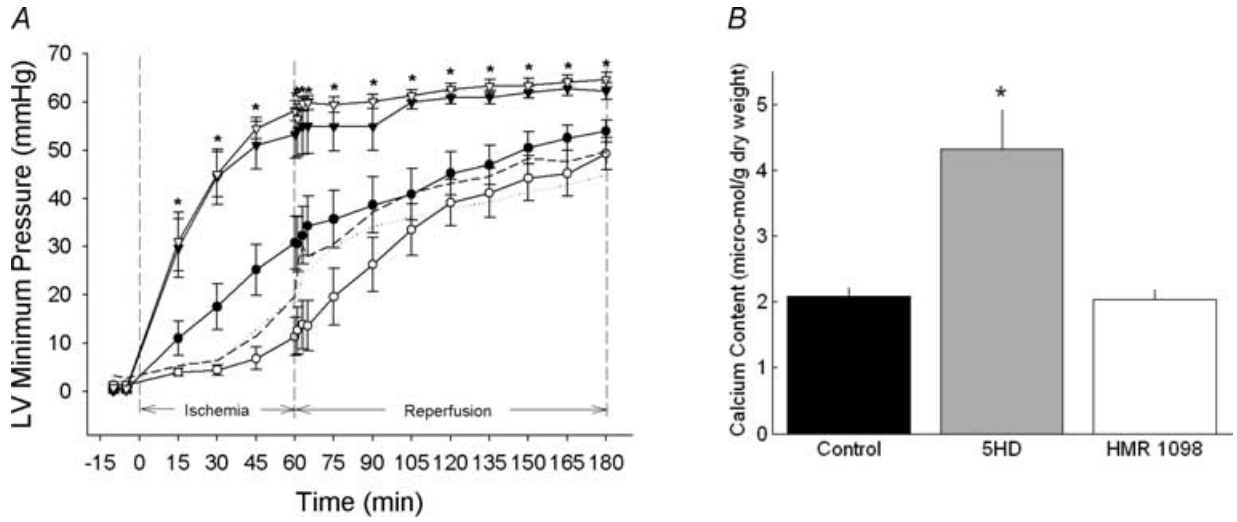

Figure 4. Left ventricular minimum pressure and calcium content during I-R
A, left ventricular minimum pressure during the ischaemia–reperfusion protocol. ●, sedentary control hearts (no channel blocker in the buffer); ○, trained control hearts (no channel blocker in the buffer); ▼ and ▽, sedentary and trained hearts, respectively, exposed to buffer with 5HD acid, a mitochondrial K_{ATP} channel blocker. $^*P < 0.05$ *versus* all other hearts exposed to either no drug or HMR 1098, a selective sarcolemmal K_{ATP} antagonist. Since there were no differences in LV minimum pressure between control hearts and those that received HMR 1098, LV minimum pressure for hearts exposed to HMR 1098 are presented as lines for sedentary (dashed line) and trained (dotted line) animals in the interest of presentational clarity. *B*, intracellular Ca^{2+} content for hearts in the study pooled as a function of drug in the perfusate. $^*P < 0.05$ *versus* all other groups.

reperfusion. LVDP dropped quickly and significantly in hearts exposed to 5HD when compared with all other groups, with a significant difference at 15 min of ischaemia that lasted throughout the protocol ($P < 0.05$). As there were no differences in systolic pressure between groups from baseline (−5 min) throughout the duration of the protocol, all changes in LVDP can be explained by significant increases in LV minimum pressure generation (Fig. 4*A*).

The addition of 5HD caused a marked decrease in LVDP in the absence of ischaemia. The drop in pressure development in non-ischaemic time-control hearts exposed to 5HD was comparable with that seen in hearts that underwent regional I–R (Fig. 5). Both Tr5HD and Sed5HD hearts displayed a marked diminution in pressure development (due to an increase in left ventricular minimum pressure) from 30 min of ischaemia throughout the duration of the protocol (Fig. 5).

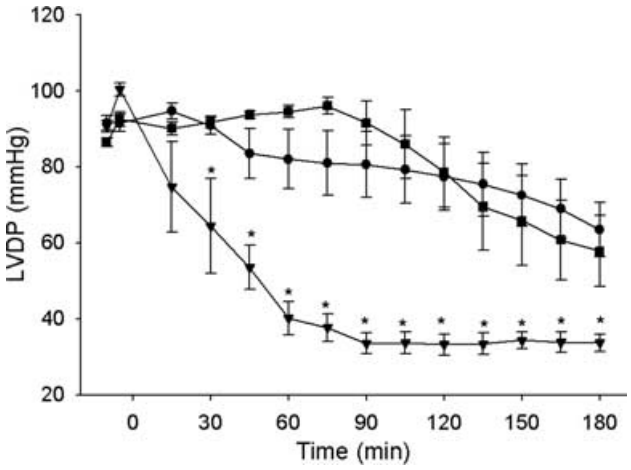

Figure 5. LVDP for non-ischaemic time-control hearts in the study
A subset of hearts was exposed to 3 h of Langendorff perfusion in the absence of ischaemia with buffer containing no drug (●), sarcolemmal K_{ATP} blocker HMR 1098 (■), and mitochondrial K_{ATP} blocker 5HD (▼). $^*P < 0.05$ *versus* all other groups.

Intracellular Ca^{2+} content

Intracellular Ca^{2+} content data are presented in Fig. 4*B*. A significant effect of drug on intracellular Ca^{2+} content was observed, with no effect of training. Accordingly, data were pooled as a function of perfused drug for presentational clarity. There was no difference in intracellular Ca^{2+} content between hearts exposed to control buffer (2.1 ± 0.1 μmol Ca^{2+} (g dry weight)$^{-1}$) or HMR 1098 (2.0 ± 0.1 μmol Ca^{2+} (g dry weight)$^{-1}$). The presence of 5HD in the perfusate caused a significant increase in intracellular Ca^{2+} content following I–R (4.3 ± 0.6 μmol Ca^{2+} (g dry weight)$^{-1}$); $P < 0.05$ when compared with control and HMR 1098).

Akt and GSK-3β phosphorylation

pAkt/Akt and pGSK-3β/GSK-3β data are presented in Fig. 6*A* and *B*, respectively. There were no differences

in content or phosphorylation of either Akt or GSK-3β between Tr and Sed hearts (P = NS).

Discussion

Role of K_{ATP} channels in exercise-induced infarct sparing

This study was conducted to determine the influence of K_{ATP} channels on exercise-induced reductions in myocardial infarct size and improvements in haemodynamic variables. Our previous work (Brown *et al.* 2003) indicated that 20 weeks of chronic exercise training caused a 25% reduction in myocardial infarct size in female rats. The present findings corroborate our previous observation, with the Tr animals having a 21% reduction in myocardial infarct size when compared to hearts from Sed animals (Fig. 1).

While the terminal mechanisms underlying exercise-induced protection from infarction are unidentified, we speculated that the involvement of myocardial K_{ATP} channels was central to the protective phenotype. MitoK_{ATP} blockade with 5HD minutes before ischaemia has been shown to negate the delayed phase of preconditioning afforded by a number of stimuli (see Table 2). Since exercise-induced PC has been proposed to share common pathways with other triggers of delayed PC (Bolli, 2000), we hypothesized that the cellular pathway(s) would be similar (i.e. also involve the activity of the mitoK_{ATP} channel). In the present study, 5HD was effective in reversing the infarct-sparing effect of 'classic' ischaemic preconditioning (see Methods); however, it did not abolish acquired protection from infarction in the exercised animals (Fig. 1). Training-induced infarct sparing was conserved in the presence of 5HD, indicating that unlike all other known stimuli of delayed PC, protection mediated by chronic exercise cannot be ascribed to mitoK_{ATP} channel activity. This finding is novel insofar as it is the first demonstration that delayed preconditioning can be conserved in the presence of mitoK_{ATP} blockade, and may reflect a novel mechanism for delayed cardioprotection that is also sustainable over time.

Recent data from our laboratory indicated that short-term exercise led to reductions in myocardial infarct size following I–R and correlated with increased expression of sarcK_{ATP} channels (Brown *et al.* 2005). The present study demonstrates that the exercise-induced increase in sarcK_{ATP} channel expression is sustainable following months of training, with chronic exercise resulting in significant increases in both K_{ir}6.2 and SUR2a, the pore-forming and regulatory subunits of the sarcolemmal channel, respectively (Fig. 2). The positive correlation between sarcK_{ATP} expression and cardioprotection that we observed in this study is congruent with findings from a number of publications where increased sarcK_{ATP} channel expression due to female gender (Ranki *et al.* 2001; Brown *et al.* 2005), oestrogen supplementation (Ranki *et al.* 2002), chronic mild hypoxia (Crawford *et al.* 2003), and hypoxic preconditioning (Budas *et al.* 2004) was also associated with cardioprotection.

A cytoprotective role for sarcK_{ATP} channels is confirmed by pharmacological blockade of the channels with HMR 1098, a specific sarcK_{ATP} channel antagonist (Gogelein

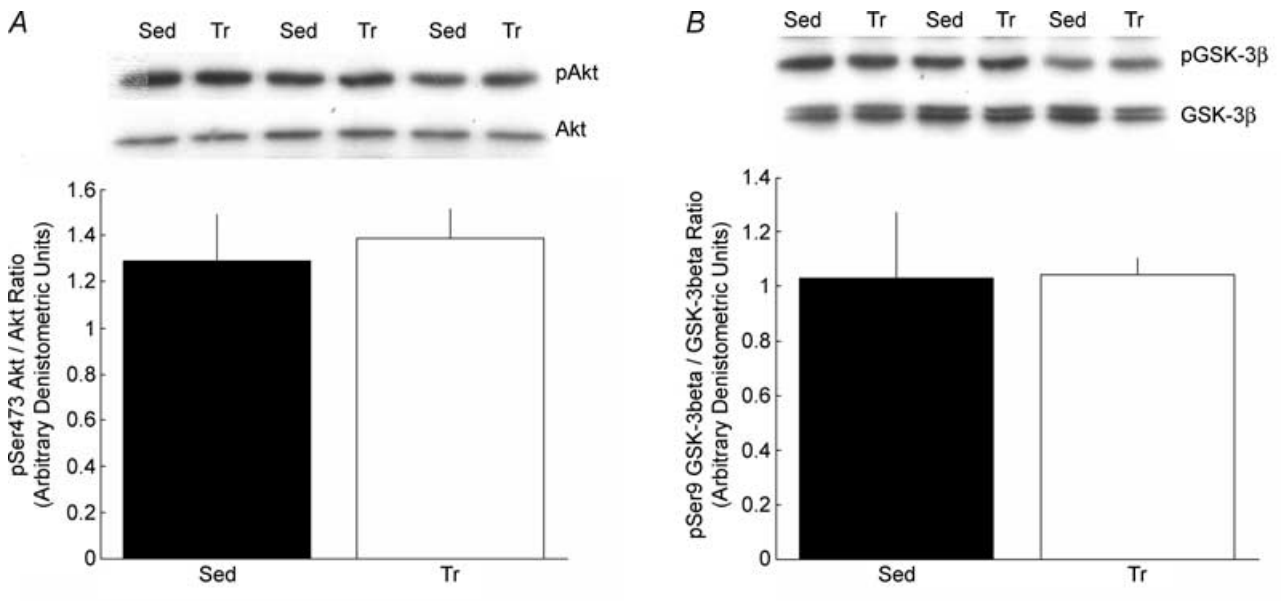

Figure 6. Myocardial Akt and GSK 3β expression
Myocardial Akt (*A*) and glycogen synthase kinase (GSK)-3β (*B*) phosphorylation ratios and representative blots from trained and sedentary animals. There were no differences in basal Akt or GSK-3β phosphorylation between training groups (P = NS).

Table 2. Summary of K_{ATP} channel contribution to delayed protection against infarction from a number of experimental stimuli

Stimlus for delayed protection	5HD sensitive?	Glibenclamide sensitive?	HMR 1098 sensitive?	Reference
Ischaemia	Yes	Yes[1]; –[2,3]	No[3]; –[1,2]	[1]Bernado *et al.* (1999*a*) [2]Takano *et al.* (2000) [3]Patel *et al.* (2005)
Adenosine receptor agonist	Yes	Yes [1,2]; –[3]	–	[1]Baxter & Yellon (1999) [2]Bernardo *et al.* (1999*b*) [3]Takano *et al.* (2001)
Heat stress	Yes	Yes	–	Hoag *et al.* (1997) Pell *et al.* (1997)
Diazoxide	Yes	–	–	Ockaili *et al.* (1999)
δ-Opioid receptor agonist	Yes	Yes	–	Fryer *et al.* (1999)
κ-Opioid receptor agonist	Yes	Yes	No	Chen *et al.* (2003)
Monophospholyl Lipid A	Yes	Yes	–	Mei *et al.* (1996)
Phosphodiesterase inhibition	Yes	–	–	Ockaili *et al.* (2002)
Succinate dehydrogenase inhibition	Yes	–	–	Ockaili *et al.* (2001)
Exercise	No	–	Yes	Herein

5HD, 5-hydroxydecanoic acid used to selectively block the mitochondrial K_{ATP} channel minutes before the onset of ischaemia; glibenclamide, a non-specific K_{ATP} channel blocker administered minutes before index ischaemia; HMR 1098, used to selectively block the sarcolemmal K_{ATP} channel minutes before the onset of ischaemia; –, not determined in the study.

et al. 1998). Administration of HMR 1098 during the I–R protocol completely abolished training-induced reductions in infarct size and led to dramatic increases in infarcted area regardless of training status (Fig. 1). Infarct size in Tr animals doubled with sarcK_{ATP} blockade and increased by 43% in Sed animals, implicating an important role for sarcK_{ATP} channel opening in myocardial protection during I–R. While few other studies have examined the role of sarcK_{ATP} channels in mediating delayed protection from infarction, these channels cannot be discounted in many protocols of acquired protection where glibenclamide, a non-specific channel antagonist, was administered minutes before the onset of ischaemia (see Table 2) and abolished the prophylactic phenotype. Furthermore, as pharmacological agents may have non-specific effects, it is important to note that the findings of this study are consistent with observations from genetic knockout studies where $K_{ir}6.2$-deficient mice lacking intact sarcK_{ATP} channels display severe intolerance and myocardial injury following the metabolic stress of chronic exercise training (Zingman *et al.* 2002; Kane *et al.* 2004, 2005).

To the best of our knowledge, chronic ethanol ingestion is the only other stimulus besides exercise training that provides sustainable (over months) protection against myocardial I–R injury (Pagel *et al.* 2000, 2002). Interestingly, a cardioprotective role of the sarcK_{ATP} channel was also confirmed in previous experiments using both specific (Pagel *et al.* 2002) and non-specific (Pagel *et al.* 2000) sarcK_{ATP} channel blockers to eliminate the protection afforded by ethanol consumption. In order to further elucidate mechanistic homology between sustainable protection afforded by ethanol and exercise training, we examined the constitutive phosphorylation of Akt and its substrate GSK-3β. While significant increases in basal Akt and GSK-3β activity have been observed following months of ethanol consumption (Zhou *et al.* 2002), there was no difference in Akt or GSK-3β expression or phosphorylation following exercise training (Fig. 6). These data indicate that both forms of sustainable protection rely on the activity of sarcK_{ATP} channels, and that other potential mediators such as activated Akt (Zhou *et al.* 2002) or mitoK_{ATP} channel activity (Pagel *et al.* 2002) may be model specific and are not obligatory for sustainable protection *per se*.

Although the precise mechanism by which a training-induced increase in sarcK_{ATP} channels would lead to tissue salvage is not known, several possibilities exist. The increased channel expression may improve the metabolic-sensing capability of the cell. During times of metabolic stress (i.e. ischaemia), increased channel number may help the cell better maintain energetic status. Genetic knockout of $K_{ir}6.2$ has previously been shown to hinder preconditioning-mediated protection of myocardial bioenergetics following I–R (Gumina *et al.* 2003; Alekseev *et al.* 2005). Furthermore, the physical association of several enzymes, including adenlyate kinase (Carrasco *et al.* 2001), creatine kinase (Crawford *et al.* 2002*b*), glyceraldehyde 3-phosphate dehydrogenase (Jovanovic *et al.* 2005), and lactate dehydrogenase (Crawford *et al.* 2002*a*), with sarcK_{ATP} channels has been previously observed. Increased channel–enzyme complexes may provide increased sensitivity for maintaining cellular energy status, with improved communication between the cell surface and

the mitochondria through the creatine kinase signalling system (Dzeja & Terzic, 1998; Sasaki *et al.* 2001; Alekseev *et al.* 2005). An increase in channel expression following exercise (Brown *et al.* 2005; herein) may be related to better preservation of ATP levels during ischaemia (Jew & Moore, 2001) as well as prolonged time course for K_{ATP} current expression (Jew & Moore, 2002) in hearts from trained animals, both of which were previously observed by our laboratory. Another possibility is that the sarcK_{ATP} channel may be a part of a pro-survival kinase pathway transduction system that decreases the likelihood of the permeability transition pore opening and the onset of apoptosis (Hausenloy & Yellon, 2004; Hausenloy *et al.* 2004, 2005). Obviously, the precise role of such protective mechanisms in exercise-induced increases in sarcK_{ATP} channels and protection from infarction warrants further investigation.

LVDP

Our observation of training-induced preservation of mechanical pressure development during I–R (Fig. 3) confirms findings from a well-documented body of literature (Scheuer & Stezoski, 1972; Bowles *et al.* 1992; Powers *et al.* 1998; Moore & Palmer, 1999; Brown *et al.* 2003; Hamilton *et al.* 2003), although the precise mechanisms involved in this phenomenon are not known. Another novel aspect of the present study was that training-induced improvements in LVDP (Fig. 3) were completely abolished with 5HD, implicating a potential role of the mitoK_{ATP} channel as a mediator of sustained ischaemic myocardial function in tissue outside of the ZAR in trained animals (as ischaemic tissue generates diminutive amounts of mechanical force). Since the molecular identity of the myocardial mitoK_{ATP} channel is not known, there could be a number of hypothetical mechanisms explaining how exercise-induced sustenance of LVDP involves the activity of this channel. Recent evidence indicates that the mitoK_{ATP} channel may belong to a multiprotein mitochondrial complex containing succinate dehydrogenase (Ardehali *et al.* 2004). Training-induced alterations in mitochondrial energy metabolism may underlie the improved ischaemic function that was abolished by 5HD. Obviously a scenario such as this is purely speculative at present, and requires much more investigation into both the basic molecular identity of this channel, as well as channel involvement in training-induced protection from mechanical dysfunction during I–R.

The fact that 5HD diminished LVDP so profoundly across groups warrants comment. The decrease in LVDP with the addition of 5HD can be explained by our observation that this drug significantly increased LV minimum pressure generation whether hearts were exposed to ischaemia (Fig. 4*A*) or not (Fig. 5). A plausible explanation for this drug-related impaired myocardial relaxation is that intracellular Ca^{2+} content was significantly increased only in hearts exposed to 5HD during I–R (Fig. 4*B*). Despite the observation that 5HD completely abolished LVDP from 15 min of ischaemia throughout the duration of the protocol, the drug did not influence infarct size or abolish training-induced protection against infarction. One possible explanation is as follows: during ischaemia, when a significant part of the LV (approx 45%) becomes ischaemic, the part of the heart *receiving* flow generates the vast majority of pressure. Although the presence of 5HD did not influence the tissue in the ZAR (since training-induced reductions in infarct size were maintained with 5HD), it drastically reduced the pressure-generating capability of the non-ischaemic region of the heart (as well as in non-ischaemic hearts; Fig. 5). Notwithstanding non-specific targets of 5HD (addressed below), these findings may implicate a role of mitoK_{ATP} channels in normoxic excitation–contraction coupling.

The observation that 5HD did not uniformly abrogate both training-induced infarct sparing and protection from mechanical dysfunction is consistent with results from other laboratories (Takano *et al.* 2000). Similarly, blockade of sarcolemmal K_{ATP} channels did not influence pressure generation in tissue outside of the ZAR, but had a very deleterious effect on tissue survival in the ischaemic region. Collectively, these data indicate that separate pathways may be involved in infarct sparing *versus* protection from stunning in delayed cardioprotection as suggested by Bolli (2000). Also of interest was our finding that 5HD caused a twofold increase in intracellular Ca^{2+} content that correlated with impaired relaxation, yet in spite of these changes in intracellular Ca^{2+} infarct size was not affected by 5HD. Furthermore, HMR 1098 in the perfusate caused marked increases in infarct size without affecting cellular Ca^{2+} homeostasis. While both $K_{ir}6.2$-genetic-knockout (Zingman *et al.* 2002) and channel-blockade (Light *et al.* 2001; Ranki *et al.* 2002) studies propose that sarcK_{ATP} channels are obligatory for protection against I–R-induced Ca^{2+} overload and subsequent tissue injury, previous work has shown that increases in intracellular Ca^{2+} alone are not sufficient to cause irreversible tissue damage (Wagenknecht *et al.* 1994). It is likely that confounding factors such as decreased pH or adenine nucleotide depletion, as well as intracellular Ca^{2+} compartmentation, may explain the tissue damage that we observed with sarcK_{ATP} blockade in the absence of elevated intracellular Ca^{2+} content.

Potential limitations

One putative limitation of the current study involves the use of 5HD to block mitoK_{ATP} channels. While 5HD has been extensively used over the last ∼10 years as a

mitochondria-specific K_{ATP} channel blocker (see reviews O'Rourke, 2000, 2004; Yellon & Downey, 2003), recent evidence questions the pharmacological specificity of this channel antagonist (Hanley *et al.* 2002, 2003, 2004; Lim *et al.* 2002). In a series of studies, Hanley *et al.* (Hanley *et al.* 2002, 2003, 2004) demonstrated that 5HD can be metabolized in heart mitochondria and may have important effects on substrate utilization independent of K_{ATP} channel activity. As suggested by these studies, 5HD may create a 'bottle neck' in the mitochondria and impair fatty acid metabolism. Decreased substrate availability would then reduce the capacity of the myocardium to perform work. This theory is supported by the observation that the same concentration of 5HD that was used herein also inhibited respiration in isolated mitochondria (Lim *et al.* 2002). Despite the possible non-specificity of 5HD, previous work has shown this drug to be effective at abrogating the beneficial effects of delayed preconditioning in a wide variety of protocols (see Table 2). A major finding of the present study is that chronic exercise training sustained delayed cardioprotection even in the presence of 5HD. To the best of our knowledge this is the first preconditioning stimulus where the 'second window' of protection from infarction is not 'closed' by 5HD.

In summary, we have demonstrated that chronic exercise training reduces myocardial infarct size following I–R in rat heart, and that this cardioprotective phenotype is characterized by an increased expression of the sarcK_{ATP} channel. Pharmacological blockade of the sarcK_{ATP} channels abrogated the training-induced protection from infarction, while mitochondrial K_{ATP} channel blocker had no effect on myocardial infarct size or training-induced preservation of LV tissue after I–R. These data indicate that the activity and/or expression of the sarcolemmal K_{ATP} channel is an obligatory part of a mechanism conferring protection from I–R injury, and that exercise training reduces myocardial infarct size by manipulating this mechanism and further increasing sarcolemmal K_{ATP} channel expression. These findings may have important clinical implications in that we have identified a cardioprotective stimulus that confers protection that is sustainable over long periods of time. Further investigation will be needed to determine the cardioprotective efficacy of an exercise intervention on a diseased population, specifically on patients who are taking sulphonylurea medication or who have genetically defective sarcK_{ATP} channels.

References

Alekseev AE, Hodgson DM, Karger AB, Park S, Zingman LV & Terzic A (2005). ATP-sensitive K^+ channel channel/enzyme multimer: metabolic gating in the heart. *J Mol Cell Cardiol* **38**, 895–905.

Alto LE & Dhalla NS (1979). Myocardial cation contents during induction of calcium paradox. *Am J Physiol Heart Circ Physiol* **237**, H713–719.

Ardehali H, Chen Z, Ko Y, Mejia-Alvarez R & Marban E (2004). Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K^+ channel activity. *Proc Natl Acad Sci U S A* **101**, 11880–11885.

Baxter GF & Ferdinandy P (2001). Delayed preconditioning of myocardium: current perspectives. *Basic Res Cardiol* **96**, 329–344.

Bernardo NL, D'Angelo M, Okubo S, Joy A & Kukreja RC (1999). Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in the rabbit heart. *Am J Physiol* **276**, H1323–1330.

Baxter GF & Yellon DM (1999*b*). ATP-sensitive K^+ channels mediate the delayed cardioprotective effect of adenosine A1 receptor activation. *J Mol Cell Cardiol* **31**, 981–989.

Bernardo NL, Okubo S, Maaieh MM, Wood MA & Kukreja RC (1999). Delayed preconditioning with adenosine is mediated by opening of ATP-sensitive K^+ channels in rabbit heart. *Am J Physiol Heart Circ Physiol* **277**, H128–135.

Bolli R (2000). The late phase of preconditioning. *Circ Res* **87**, 972–983.

Bowles DK, Farrar RP & Starnes JW (1992). Exercise training improves cardiac function after ischemia in the isolated, working rat heart. *Am J Physiol Heart Circ Physiol* **263**, H804–809.

Brown DA, Jew KN, Sparagna GC, Musch TI & Moore RL (2003). Exercise training preserves coronary flow and reduces infarct size following ischemia–reperfusion in rat heart. *J Appl Physiol* **95**, 2510–2518.

Brown DA, Lynch JM, Armstrong CA, Caruso NM, Ehlers LB, Johnson MS *et al.* (2005). Susceptibility of the heart to ischaemia–reperfusion injury and exercise-induced cardioprotection are sex-dependent. *J Physiol* **564**, 619–630.

Budas GR, Jovanovic S, Crawford RM & Jovanovic A (2004). Hypoxia-induced preconditioning in adult stimulated cardiomyocytes is mediated by the opening and trafficking of sarcolemmal KATP channels. *Faseb J* **18**, 1046–1048.

Carrasco AJ, Dzeja PP, Alekseev AE, Pucar D, Zingman LV, Abraham MR *et al.* (2001). Adenylate kinase phosphotransfer communicates cellular energetic signals to ATP-sensitive potassium channels. *Proc Natl Acad Sci U S A* **98**, 7623–7628.

Chen M, Zhou JJ, Kam KW, Qi JS, Yan WY, Wu S *et al.* (2003). Roles of KATP channels in delayed cardioprotection and intracellular Ca^{2+} in the rat heart as revealed by kappa-opioid receptor stimulation with U50488H. *Br J Pharmacol* **140**, 750–758.

Cohen MV, Yang XM & Downey JM (1994). Conscious rabbits become tolerant to multiple episodes of ischemic preconditioning. *Circ Res* **74**, 998–1004.

Crawford RM, Budas GR, Jovanovic S, Ranki HJ, Wilson TJ, Davies AM *et al.* (2002*a*). M-LDH serves as a sarcolemmal K(ATP) channel subunit essential for cell protection against ischemia. *EMBO J* **21**, 3936–3948.

Crawford RM, Jovanovic S, Budas GR, Davies AM, Lad H, Wenger RH *et al.* (2003). Chronic mild hypoxia protects heart-derived H9c2 cells against acute hypoxia/reoxygenation by regulating expression of the SUR2A subunit of the ATP-sensitive K^+ channel. *J Biol Chem* **278**, 31444–31455.

Crawford RM, Ranki HJ, Botting CH, Budas GR & Jovanovic A (2002*b*). Creatine kinase is physically associated with the cardiac ATP-sensitive K^+ channel *in vivo*. *Faseb J* **16**, 102–104.

D'Souza SP, Yellon DM, Martin C, Schulz R, Heusch G, Onody A *et al.* (2003). B-type natriuretic peptide limits infarct size in rat isolated hearts via KATP channel opening. *Am J Physiol Heart Circ Physiol* **284**, H1592–1600.

Dzeja PP & Terzic A (1998). Phosphotransfer reactions in the regulation of ATP-sensitive K^+ channels. *Faseb J* **12**, 523–529.

Fryer RM, Hsu AK, Eells JT, Nagase H & Gross GJ (1999). Opioid-induced second window of cardioprotection: potential role of mitochondrial KATP channels. *Circ Res* **84**, 846–851.

Gogelein H, Hartung J, Englert HC & Scholkens BA (1998). HMR 1883a novel cardioselective inhibitor of the ATP-sensitive potassium channel. Part I: effects on cardiomyocytes, coronary flow and pancreatic beta-cells. *J Pharmacol Exp Ther* **286**, 1453–1464.

Gumina RJ, Pucar D, Bast P, Hodgson DM, Kurtz CE, Dzeja PP *et al.* (2003). Knockout of Kir6.2 negates ischemic preconditioning-induced protection of myocardial energetics. *Am J Physiol Heart Circ Physiol* **284**, H2106–2113.

Hamilton KL, Staib JL, Phillips T, Hess A, Lennon SL & Powers SK (2003). Exercise, antioxidants, and HSP72: protection against myocardial ischemia/reperfusion. *Free Radic Biol Med* **34**, 800–809.

Hanley PJ, Drose S, Brandt U, Lareau RA, Banerjee AL, Srivastava DK *et al.* (2005). 5-hydroxydecanoate is metabolised in mitochondria and creates a rate-limiting bottleneck for β-oxidation of fatty acids. *J Physiol* **562**, 307–318.

Hanley PJ, Gopalan KV, Lareau RA, Srivastava DK, von Meltzer M & Daut J (2003). Beta-oxidation of 5-hydroxydecanoate, a putative blocker of mitochondrial ATP-sensitive potassium channels. *J Physiol* **547**, 387–393.

Hanley PJ, Mickel M, Loffler M, Brandt U & Daut J (2002). K(ATP) channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart. *J Physiol* **542**, 735–741.

Hausenloy DJ, Tsang A, Mocanu MM & Yellon DM (2005). Ischemic preconditioning protects by activating prosurvival kinases at reperfusion. *Am J Physiol Heart Circ Physiol* **288**, H971–976.

Hausenloy DJ & Yellon DM (2004). New directions for protecting the heart against ischaemia–reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK) pathway. *Cardiovasc Res* **61**, 448–460.

Hausenloy DJ, Yellon DM, Mani-Babu S & Duchen MR (2004). Preconditioning protects by inhibiting the mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol* **287**, H841–849.

Herlitz J, Bengtson A, Hjalmarson A & Karlson BW (1988). Morbidity during five years after myocardial infarction and its relation to infarct size. *Clin Cardiol* **11**, 672–677.

Hoag JB, Qian YZ, Irageem MA, D'Angelo M & Kukreja RC (1997). ATP-sensitive potassium channel mediates delayed ischemic protection by heat stress in rabbit heart. *Am J Physiol* **273**, H2458–2464.

Jew KN & Moore RL (2001). Glibenclamide improves postischemic recovery of myocardial contractile function in trained and sedentary rats. *J Appl Physiol* **91**, 1545–1554.

Jew KN & Moore RL (2002). Exercise training alters an anoxia-induced, glibenclamide-sensitive current in rat ventricular cardiocytes. *J Appl Physiol* **92**, 1473–1479.

Jovanovic S, Du Q, Crawford RM, Budas GR, Stagljar I & Jovanovic A (2005). Glyceraldehyde 3-phosphate dehydrogenase serves as an accessorg protein of the cardiac sarcolemmal K(ATP) channel. *EMBO Report*.

Kane GC, Behfar A, Yamada S, Perez-Terzic C, O'Cochlain F, Reyes S *et al.* (2004). ATP-sensitive K^+ channel knockout compromises the metabolic benefit of exercise training, resulting in cardiac deficits. *Diabetes* **53** (Suppl. 3), S169–S175.

Kane GC, Liu XK, Yamada S, Olson TM & Terzic A (2005). Cardiac KATP channels in health and disease. *J Mol Cell Cardiol* **38**, 937–943.

Kehl F, Krolikowski JG, LaDisa JF Jr, Kersten JR, Warltier DC & Pagel PS (2003). Adenosine type 1 (A1) receptors mediate protection against myocardial infarction produced by chronic, intermittent ingestion of ethanol in dogs. *Int J Cardiol* **88**, 175–182.

Kristiansen SB, Henning O, Kharbanda RK, Nielsen-Kudsk JE, Schmidt MR, Redington AN *et al.* (2005). Remote preconditioning reduces ischemic injury in the explanted heart by a KATP channel-dependent mechanism. *Am J Physiol Heart Circ Physiol* **288**, H1252–1256.

Light PE, Kanji HD, Fox JE & French RJ (2001). Distinct myoprotective roles of cardiac sarcolemmal and mitochondrial KATP channels during metabolic inhibition and recovery. *Faseb J* **15**, 2586–2594.

Lim KH, Javadov SA, Das M, Clarke SJ, Suleiman MS & Halestrap AP (2002). The effects of ischaemic preconditioning, diazoxide and 5-hydroxydecanoate on rat heart mitochondrial volume and respiration. *J Physiol* **545**, 961–974.

Mei DA, Elliot GT & Gross GJ (1996). KATP channels mediate late preconditioning against intaraction produced by monophosphoryl lipid A. *Am J Physiol* **271**, H2723–2729.

Miller TD, Christian TF, Hopfenspirger MR, Hodge DO, Gersh BJ & Gibbons RJ (1995). Infarct size after acute myocardial infarction measured by quantitative tomographic 99mTc sestamibi imaging predicts subsequent mortality. *Circulation* **92**, 334–341.

Moore RL & Palmer BM (1999). Exercise training and cellular adaptations of normal and diseased hearts. *Exerc Sport Sci Rev* **27**, 285–315.

Moraska A, Deak T, Spencer RL, Roth D & Fleshner M (2000). Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *Am J Physiol Regul Integr Comp Physiol* **279**, R1321–1329.

Murry CE, Jennings RB & Reimer KA (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**, 1124–1136.

National Health and Nutrition Examination Survey III (2000). *Heart Attack and Angina Statistics*, Centers for Disease Control and Prevention, Hyattsville, MD.

O'Rourke B (2000). Myocardial K (ATP) channels in preconditioning. *Circ Res* **87**, 845–855.

O'Rourke B (2004). Evidence for mitochondrial K^+ channels and their role in cardioprotection. *Circ Res* **94**, 420–432.

Ockaili R, Emani VR, Okubo S, Brown M, Krottupalli K & Kukreja RC (1999). Opening of mitochondrial KATP channel induces early and delayed cardioprotective oxide: role of nitric oxide. *Am J Physiol* **277**, H2425–2434.

Ockaili RA, Bhargava P & Kukreja RC (2001). Chemical preconditioning with 3-nitropropionic acid in hearts: role of mitochondrial K(ATP) channel. *Am J Physiol Heart Circ Physiol* **280**, H2406–2411.

Ockaili R, Salloum F, Hawkins J & Kukreja RC (2002). Sildenafil citrate (viagra) induces powerful cardioprotective effect via opening of mitochondrial K(ATP) channels in rabbits. *Am J Physiol Heart Circ Physiol* **283**, H1263–1269.

Pagel PS, Krolikowski JG, Kehl F, Mraovic B, Kersten JR & Warltier DC (2002). The role of mitochondrial and sarcolemmal K(ATP) channels in canine ethanol-induced preconditioning in vivo. *Anesth Analg* **94**, 841–848.

Pagel PS, Toller WG, Gross ER, Gare M, Kersten JR & Warltier DC (2000). K(ATP) channels mediate the beneficial effects of chronic ethanol ingestion. *Am J Physiol Heart Circ Physiol* **279**, H2574–2579.

Pell TJ, Yellon DM, Goodwin RW & Baxter GF (1997). Myocardial ischemic tolerance following heat stress is abolished by ATP-sensitive potassium channel blockade. *Cardiovasc Drugs Ther* **11**, 679–686.

Patel IIH, Gross ER, Peart JN, Hso AK & Gross GJ (2005). Sarcolemmal KATP channel triggers delayed ischemic preconditioning in rats. *Am J Physiol Heart Circ Physiol* **288**, H445–447.

Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL *et al.* (1998). Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. *Am J Physiol Regul Integr Comp Physiol* **275**, R1468–1477.

Ranki HJ, Budas GR, Crawford RM, Davies AM & Jovanovic A (2002). 17Beta-estradiol regulates expression of K(ATP) channels in heart-derived H9c2 cells. *J Am Coll Cardiol* **40**, 367–374.

Ranki HJ, Budas GR, Crawford RM & Jovanovic A (2001). Gender-specific difference in cardiac ATP-sensitive K^+ channels. *J Am Coll Cardiol* **38**, 906–915.

Sasaki N, Sato T, Marban E & O'Rourke B (2001). ATP consumption by uncoupled mitochondria activates sarcolemmal K (ATP) channels in cardiac myocytes. *Am J Physiol Heart Circ Physiol* **280**, H1882–1888.

Scheuer J & Stezoski SW (1972). Effect of physical training on the mechanical and metabolic response of the rat heart to hypoxia. *Circ Res* **30**, 418–429.

Selye H (1998). A syndrome produced by diverse nocuous agents 1936. *J Neuropsychiatry Clin Neurosci* **10**, 230–231.

Takano H, Tang XL & Bolli R (2000). Differential role of K(ATP) channels in late preconditioning against myocardial stunning and infarction in rabbits. *Am J Physiol Heart Circ Physiol* **279**, H2350–2359.

Takano H, Bolli R, Black RG, Jr., Kodani E, Tang XL, Yang Z *et al.* (2001). A(1) or A(3) adenosine receptors induce late preconditioning against infarction in conscious rabbits by different mechanisms. *Circ Res* **88**, 520–528.

Tsuchida A, Thompson R, Olsson RA & Downey JM (1994). The anti-infarct effect of an adenosine A1-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischaemic preconditioning in rabbit heart. *J Mol Cell Cardiol* **26**, 303–311.

Wagenknecht B, Freudenrich CC, LeFurgey A & Lieberman M (1994). Calcium depletion and repletion in cultured chick heart muscle cells. *J Mol Cell Cardiol* **26**, 797–808.

Yamashita N, Baxter GF & Yellon DM (2001). Exercise directly enhances myocardial tolerance to ischaemia-reperfusion injury in the rat through a protein kinase C mediated mechanism. *Heart* **85**, 331–336.

Yamashita N, Hoshida S, Otsu K, Asahi M, Kuzuya T & Hori M (1999). Exercise provides direct biphasic cardioprotection via manganese superoxide dismutase activation. *J Exp Med* **189**, 1699–1706.

Yellon DM & Downey JM (2003). Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* **83**, 1113–1151.

Zhou HZ, Karliner JS & Gray MO (2002). Moderate alcohol consumption induces sustained cardiac protection by activating PKC-epsilon and Akt. *Am J Physiol Heart Circ Physiol* **283**, H165–174.

Zingman LV, Hodgson DM, Bast PH, Kane GC, Perez-Terzic C, Gumina RJ *et al.* (2002). Kir6.2 is required for adaptation to stress. *Proc Natl Acad Sci U S A* **99**, 13278–13283.

Acknowledgements

This work was supported by PHS grants HL40306 and HL72790 (R.L.M.), National Institute of Ageing Institutional Training Grant (AG 279; D.A.B. and A.J.C.), and the NIH/Howard Hughes Medical Institute Scholarship Program for Diversity (NIH GM066728-01; M.S.J.). We thank Dr Earl Noble for editorial assistance with the manuscript, and Dr Heinz Gögelein at Aventis Pharma, Frankfurt, Germany, for the gift of HMR 1098. The authors also wish to acknowledge the contributions of Mike Allender, Pat Chestnut, Mark DeBergh, Ryan Gardner, Cody Gillenwater, Kurt Marshall, Melissa Maxey, Bryce Moore, and Derek Zachman for technical assistance with the experimental animals.